# Parallel Artificial Membrane Permeability Assay: A New Membrane for the Fast Prediction of Passive Human Skin Permeability

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This work was devoted to the search for new artificial membranes allowing a rapid evaluation of passive human skin permeation of compounds with a parallel artificial membrane permeability assay (PAMPA). Effective permeability coefficients ($P_e$) determined for a set of compounds using the PAMPA technique with isopropyl myristate (IPM) and silicone oil, alone or in mixture, were compared to the corresponding human skin permeability coefficient values ($K_p$). A good correlation between $P_e$ and $K_p$ was found for compounds tested through a membrane consisting of 70% silicone and 30% IPM. Moreover, positive correlation between the membrane retention of compounds and stratum corneum/water partition coefficients ($P_{SC}$) was established. These results showed that this new artificial membrane, defined as PAMPA-skin, is able to mimic the main barrier properties of human stratum corneum and can be used for the fast prediction of passive human skin permeability coefficients.

## Introduction

The assessment of skin permeation is crucial for the estimation of the potential of transdermal drug delivery, for the evaluation of the risk associated with dermal contacts with toxic substances, and in the cosmetics industry. Although much progress has been made in the past decades, the understanding of permeation processes and the development of SAR or QSAR[a] models to interpret skin permeation are hindered by the complex nature of the skin barrier[1] mainly associated with the highly ordered intercellular lipid matrix of the stratum corneum, which is the essential layer providing excellent barrier properties.[2]

To estimate the ability of drug candidates to permeate human skin, model membranes are widely employed to measure fluxes, partition coefficients, and diffusion coefficients.[3] Although these in vitro experiments cannot fully reproduce in vivo conditions particularly with respect to metabolism, distribution, and blood supply, their major advantage is that experimental conditions can be controlled precisely such that the only variables are membranes and the tested compounds.[3,4]

The most common methods for the evaluation of in vitro skin permeation use diffusion cells.[5] The classic diffusion cell consists of donor and acceptor compartments separated by a membrane.[6,7] Although human excised skin is the membrane of choice, the relative difficulty of obtaining human skin samples and the high variability of sources have also led to the use of artificial membranes.[8] Several synthetic membranes have been used: solid membranes such as cellulose acetate[9,10] and dimethylpolysiloxane,[11−13] organic liquid-supported membranes containing hydrocarbons, long-chain alcohols, or isopropyl myristate,[7,14,15] and skin lipids.[16,17] The transport across the different membranes is usually determined by monitoring the delivery rate of chemical permeated into the receptor solution or its rate loss from the donor.[3,18,19]

Although semiautomated diffusion equipment has recently been developed,[20] the diffusion cell method still requires long experimental times for the measurements, and as a consequence, the method is unsuitable for high-throughput screening applications.

HTS is an emerging concept in the field of transdermal drug delivery, and new opportunities exist to develop new methods and formulations.[21] For example, a recent new high-throughput method for screening transdermal formulation has been developed[22,23] on the basis of skin conductivity and penetration of model radiolabeled permeants.

The parallel artificial membrane permeability assay is a recent procedure developed for a rapid determination of passive transport permeability that is gaining acceptance in pharmaceutical research.[24] In PAMPA, a 96-well filter plate coated with a liquid artificial membrane is used to separate two compartments: one containing a buffer solution of compounds to be tested (defined as donor compartment) and the other containing an initial fresh buffer solution (defined as acceptor compartment). Permeation determined with PAMPA using filters impregnated with a solution of phospholipids[24] or hexadecane[25] provided significant correlations with gastrointestinal absorption in humans. Later, Di et al.[26] proposed a modification of the PAMPA assay to predict passive transport through the blood−brain barrier using a solution of porcine polar brain lipids as the artificial membrane.

At present, no applications of PAMPA on the prediction of skin permeability have been reported. The purpose of this study was to develop a new artificial membrane to be used in PAMPA for the fast prediction of human skin permeation. Dimethylpolysiloxane (silicone) membranes have been extensively used as a simple lipid-like membrane model for in vitro percutaneous penetration studies[11,13,27,28] because this polymer alone or in combination with other polymers offers a nonporous, hydrophobic, and relatively inert reproducible barrier.[4,29] Isopropyl myristate has also been recommended for studies related to skin

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[a] Abbreviations: QSAR, quantitative structure−activity relationship; SC, stratum corneum; HTS, high-throughput screening; PAMPA, parallel artificial membrane permeability assay; IPM, isopropyl myristate; log $P_{oct}$, logarithm of the octanol/water partition coefficient; PVDF, polyvinylidene fluoride; $K_{p(sil)}$, permeability coefficient measured with a diffusion cell method using silicone membrane; $P_e$, permeability coefficient measured with PAMPA technique; $K_p$, permeability coefficient measured in vitro through human skin; log $P_{SC}$, logarithm of the stratum corneum/water partition coefficient; log $P_{alk}$, logarithm of the alkane/water partition coefficient; DMSO, dimethyl sulfoxide; $\tau_{LAG}$, lag time of diffusion.

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**Table 1.** Permeability Coefficients Obtained through Human Skin (log $K_p$) and through Silicone Membrane Using Diffusion Cells (log $K_{P(sil)}$), Effective Permeability Coefficients (log $P_e$) Obtained after 7 h of Incubation Time through 100% Silicone Membrane, 100% IPM Membrane, and 70% Silicone−30% IPM Membrane Using PAMPA for the First 19 Tested Compounds[a]

| compd | log $K_p$ [b] | log $K_{p(sil)}$ | 100% silicone log $P_e$ | 100% IPM log $P_e$ | 70% silicone−30% IPM | | |
|---|---|---|---|---|---|---|---|
| | | | | | % $R$ | % $C_A(t)/C_D(0)$ | log $P_e$ |
| caffeine | −7.56[39] | | | −5.05 ± 0.03 | <1 | 4.3 ± 0.2 | −5.63 ± 0.02 |
| antipyrine | −7.74 | | | −5.40 ± 0.06 | <1 | 1.2 ± 0.5 | −6.20 ± 0.17 |
| resorcinol | −7.18 | | | −4.66 ± 0.04 | <1 | 4.4 ± 0.8 | −5.62 ± 0.08 |
| nicotine | −5.26 | −4.29[13] | −4.68 ± 0.02 | −4.23 ± 0.05 | 4.1 ± 1.1 | 30.2 ± 4.9 | −4.58 ± 0.12 |
| 2-amino-4-nitrophenol | −6.62 | | | −3.96 ± 0.19 | <1 | 18.2 ± 0.8 | −4.84 ± 0.05 |
| phenol | −5.64 | −4.45[13] | −4.53 ± 0.05 | −4.00 ± 0.13 | <1 | 46.7 ± 0.6 | −4.14 ± 0.03 |
| phenobarbital | −6.90 | | | −4.16 ± 0.03 | <1 | 11.7 ± 0.4 | −5.15 ± 0.02 |
| hydrocortisone | −7.19[38] | | | −4.73 ± 0.01 | <1 | 2.2 ± 0.4 | −5.94 ± 0.07 |
| 4-nitrophenol | −5.81 | −5.08[13] | −5.24 ± 0.02 | −4.09 ± 0.03 | 4.6 ± 2.6 | 39.9. ± 0.9 | −4.33 ± 0.06 |
| lidocaine | −5.32[41] | −4.05[13] | −4.19 ± 0.07 | −4.33 ± 0.02 | 9.7 ± 3.3 | 36.8 ± 1.0 | −4.35 ± 0.03 |
| salycilic acid | −5.45 | −4.70 | −4.74 ± 0.01 | −3.98 ± 0.04 | 5.7 ± 1.2 | 43.1 ± 1.6 | −4.16 ± 0.02 |
| benzyl nicotinate | −5.35 | | −4.18 ± 0.05 | −4.35 ± 0.09 | 40.6 ± 1.6 | 26.0 ± 1.0 | −4.26 ± 0.02 |
| 4-bromophenol | −5.00 | −4.00[13] | −4.23 ± 0.06 | −4.08 ± 019 | 10.9 ± 0.9 | 44.1 ± 0.8 | −3.90 ± 0.08 |
| 2-naphthol | −5.11 | −4.08 | −4.35 ± 0.01 | −4.41 ± 0.02 | 20.1 ± 1.1 | 36.5 ± 0.7 | −4.19 ± 0.03 |
| ketoprofen | −4.70 | | −4.79 ± 0.02 | −4.29 ± 0.03 | 12.1 ± 1.9 | 38.5 ± 0.8 | −4.25 ± 0.01 |
| testosterone | −5.83[49] | | −4.61 ± 0.03 | −4.25 ± 0.01 | 9.2 ± 1.6 | 42.9 ± 1.0 | −4.11 ± 0.04 |
| thymol | −4.83 | −3.68 | −4.11 ± 0.05 | −4.23 ± 0.03 | 66.2 ± 3.7 | 13.9 ± 2.1 | −4.34 ± 0.06 |
| naproxen | −4.97 | | −4.51 ± 0.02 | −4.31 ± 0.05 | 23.1 ± 3.3 | 36.2 ± 1.2 | −4.11 ± 0.11 |
| progesterone | −5.08[38] | | −4.31 ± 0.05 | −4.63 ± 0.23 | 73.5 ± 2.0 | 8.6 ± 0.6 | −4.56 ± 0.06 |

[a] Membrane retention ($R$) and $C_A(t)/C_D(0)$ values obtained from 70% silicone−30% IPM membrane are also shown. Permeability coefficients ($K_p$, $K_{P(sil)}$, and $P_e$) are expressed in cm/s. [b] From ref 36.

**Table 2.** Permeability Coefficients Obtained through Human Skin (log $K_p$), Membrane Retention ($R$), $C_A(t)/C_D(0)$ Values, and Effective Permeability Coefficients (log $P_e$) Obtained after 7 h of Incubation Time through 70% Silicone−30% IPM Membrane Using PAMPA for the 11 Added Compounds[a]

| compd | log $K_p$ [b] | 70% silicone−30% IPM | | |
|---|---|---|---|---|
| | | % $R$ | % $C_A(t)/C_D(0)$ | log $P_e$ |
| 2-nitro-*p*-phenylendiamine | −6.86 | <1 | 17.4 ± 0.5 | −4.94 ± 0.02 |
| methyl nicotinate | −6.04 | 10.7 ± 0.4 | 37.2 ± 1.0 | −4.32 ± 0.06 |
| ephedrine | −5.75 | <1 | 21.8 ± 4.3 | −4.82 ± 0.12 |
| corticosterone | −7.08[39] | <1 | 13.6 ± 0.1 | −5.06 ± 0.01 |
| piroxicam | −6.02 | 7.2 ± 0.9 | 37.7 ± 0.6 | −4.35 ± 0.02 |
| dexamethasone | −7.75[49] | <1 | 3.2 ± 0.4 | −5.75 ± 0.05 |
| isoquinoline | −5.33 | 7.3 ± 4.1 | 44.5 ± 1.5 | −4.05 ± 0.04 |
| 4-chlorophenol | −5.00 | 16.8 ± 0.5 | 39.4 ± 0.8 | −4.10 ± 0.06 |
| 4-ethylphenol | −5.01 | 13.6 ± 2.3 | 41.6 ± 1.7 | −4.05 ± 0.08 |
| atrazine | −5.56 | 13.5 ± 4.8 | 38.8 ± 0.6 | −4.21 ± 0.05 |
| indomethacin | −5.39 | 52.8 ± 5.3 | 21.3 ± 2.7 | −4.20 ± 0.08 |
| diclofenac | −5.30 | 72.4 ± 4.0 | 11.0 ± 1.9 | −4.33 ± 0.11 |

[a] Permeability coefficients ($K_p$ and $P_e$) are expressed in cm/s. [b] From ref 36.

diffusion because its polar and nonpolar properties are considered to mimic, in a simple way, skin lipids.[14,30,31] For example, IPM has been used as a lipophilic phase in partitioning experiments[15,30] and as an artificial membrane in rotating diffusion cells.[7] Therefore, IPM, silicone oil, and mixtures of the two components were immobilized on filters and tested as liquid supported membranes in PAMPA to evaluate their potential to mimic the human skin barrier.

## Results and Discussion

In Tables 1−3, compounds are sorted according to log $P_{oct}$ values used as the lipophilicity indicator.

**HTS Alternative to the Diffusion Cell Method.** Liquid silicone (DC200) was immobilized on hydrophobic PVDF filter plates, and permeability through this artificial membrane was determined using the PAMPA technique. To evaluate whether PAMPA could provide the same information as diffusion cell methods regarding compounds' permeation, permeability coefficients (defined as log $K_{p(sil)}$) of eight compounds measured with a diffusion cell method published by Geinoz et al.[42] that used a silicone membrane (silastic) were compared to effective permeability coefficients (log $P_e$) obtained with PAMPA using liquid silicone membrane (Table 1).

A good correlation was found between the permeability coefficients log $P_e$ measured with PAMPA and log $K_{p(sil)}$ determined with this diffusion cell method (Figure 1) showing that PAMPA can be a high-throughput screening alternative to this diffusion cell method.

**PVDF Filters Coated with Silicone.** Nineteen compounds were tested with PAMPA using silicone membrane. Effective permeability coefficients (log $P_e$) values are shown in Table 1. The retention $R$ of compounds was negligible for most of the tested compounds except for 2-naphthol, benzyl nicotinate, progesterone, and thymol. Moreover, most of the polar compounds were not able to permeate the silicone membrane ($C_A(t)/C_D(0)$ values less than 1% were measured), and thus, the accurate determination of log $P_e$ was not possible for these compounds (see Supporting Information for $R$ and $C_A(t)/C_D(0)$ values).

The log $P_e$ values were then compared to their permeability coefficients log $K_p$ obtained from human permeation measurements (Figure 2A). Two different groups can be clearly distinguished in the graph: a group of compounds with a high permeation both through silicone membrane and through human skin (log $K_p > -6$) and a group of compounds with a very low permeation through silicone membrane and through human skin (log $K_p < -6$) (in this group the number of compounds in the acceptor compartment was not accurately UV-detectable after incubation time). The hydrophobic nature of silicone probably allows only the permeation of the more hydrophobic compounds

**Table 3.** Physicochemical Parameters (Ionization Constant p$K_a$ and Lipophilicity Values in Octanol−Water System, log $P_{Oct}$, in Alkane−Water System, log $P_{Alk}$, and in Stratum Corneum−Water System, log $P_{SC}$) of Tested Compounds

| compd | p$K_a$ | log $P_{oct}$ [a] | log $P_{alk}$ [c] | log $P_{SC}$ [d] |
|---|---|---|---|---|
| caffeine | 0.60[34] | −0.07 | | |
| antipyrine | 1.44[34] | 0.56 | | |
| resorcinol | 9.21 | 0.80 | | 0.26 |
| nicotine | 8.08/3.21[13] | 1.17 | | |
| 2-amino-4-nitrophenol | 6.70/2.88 | 1.18[b] | | |
| phenol | 9.99[13] | 1.47 | | |
| phenobarbital | 7.49[34] | 1.47 | | |
| hydrocortisone | | 1.61 | | 0.38 |
| 4-nitrophenol | 6.90[13] | 1.85[b] | −2.15 | 1.11 |
| lidocaine | 7.94[13] | 2.26 | | |
| salycilic acid | 2.88[34] | 2.26 | | |
| benzyl nicotinate | 3.94 | 2.40 | | |
| 4-bromophenol | 9.13[13] | 2.59 | −0.2 | 1.43 |
| 2-naphthol | 9.40 | 2.70 | 0.3 | 1.52 |
| ketoprofen | 4.25[50] | 3.12 | | |
| testosterone | | 3.22[b] | 0.41 | 1.00 |
| thymol | 10.60 | 3.30 | 1.40 | 1.86 |
| naproxen | 4.18[50] | 3.34 | | |
| progesterone | | 3.87 | 1.23 | 1.70 |
| 2-nitro-*p*-phenylendiamine | 4.36 | 0.75[b] | | 0.40 |
| methyl nicotinate | 3.84 | 0.87 | | |
| ephedrine | 9.65[34] | 0.93 | | |
| corticosterone | | 1.94 | −1.62 | 0.63 |
| piroxicam | 1.86/5.46[51] | 1.98 | | |
| dexamethasone | | 2.01 | | |
| isoquinoline | 5.37 | 2.08 | | |
| 4-chlorophenol | 9.26 | 2.39 | −0.12 | 1.31 |
| 4-ethylphenol | 10.21 | 2.47 | | 1.26 |
| atrazine | <2 | 2.61 | | 1.30 |
| indomethacin | 4.42[50] | 4.27 | | |
| diclofenac | 4.16 | 4.40 | | |

[a] From Medchem05 (Daylight Chemical Information System, Inc., Irvine, CA, 2005). [b] Values calculated using CLOGP, version 4.91 (Daylight Chemical Information System, Inc., Irvine, CA, 2005). [c] From ref 52. [d] From ref 48.

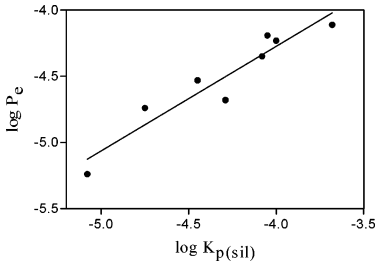


**Figure 1.** Correlation between permeability coefficients determined through PAMPA silicone artificial membrane (log $P_e$) and permeability coefficients determined through silicone membranes in diffusion cells (log $K_{p(sil)}$). The line was obtained by the linear regression equation $\log P_e = (0.79 \pm 0.11) \log K_{p(sil)} - (1.11 \pm 0.46)$, where $n = 8$, $r^2 = 0.90$, $s = 0.13$, and $F = 56$.

through the artificial membrane. It has already been reported that silicone membranes cannot be used for accurate determination of log $K_p$ for polar compounds (log $P_{oct} < 1$) with diffusion cell method[13] while for a limited set of compounds having a log $P_{oct} > 1$ (seven compounds including five phenols) a good correlation was found between $K_p$ and $K_{p(sil)}$ values. In the PAMPA experiments, for a larger set of compounds, the silicone membrane system behaved like an on/off switch. Consequently, PAMPA with silicone membrane was not able to predict $K_p$ values but may be used only to screen out compounds with high or low permeation.

**PVDF Filters Coated with IPM.** It is well-known that the stratum corneum consists of two alternating lipophilic and hydrophilic layers.[43] In this context, an artificial membrane that would present both hydrophobic and polar moieties would be preferred. Thus, IPM, having this molecular property, was immobilized on hydrophobic PVDF filter plates and the permeability through the IPM membrane using the PAMPA technique was determined for 19 compounds. Effective permeability coefficients (log $P_e$) values are shown in Table 1.

IPM membrane retention $R$ for compounds having log $P_{oct} < 2.3$ was low (<18%) and even negligible with IPM coated filters. However, the most hydrophobic compounds (log $P_{oct} \geq 2.3$) presented a considerable retention (>40%) on IPM. Membrane permeation ($C_A(t)/C_D(0)$) was high for the compounds having low or negligible retention and was low for the most lipophilic compounds (see Supporting Information for $R$ and $C_A(t)/C_D(0)$ values).

Permeability coefficients determined through IPM membrane (log $P_e$) were also compared to those determined through human skin (log $K_p$) (Figure 2B). A low log $P_e$ discrimination toward log $K_p$ and a poor correlation between the two permeation coefficients were observed. These results demonstrate that pure IPM membrane is not a suitable skinlike model in PAMPA.

**PVDF Filters Coated with Silicone−IPM Mixtures.** The results above showed that silicone membrane behaves like an overselective system, while IPM membrane provides a very poor discrimination in terms of the compounds' log $P_e$ values. The addition of IPM in fully hydrophobic silicone of IPM presenting both polar and nonpolar moieties increases the polarity of system. Therefore, permeation of 19 compounds through new membranes composed of varying percentages of IPM and silicone was studied. Figure 2 shows the relationship between permeability coefficients determined through the human skin (log $K_p$) and those determined with PAMPA through silicone−IPM membranes (log $P_e$). The addition of IPM to silicone modified the membrane properties, boosting the permeability of most of them, especially those having lower $K_p$.

Two different groups of compounds were still clearly distinguished with the addition of 10% of IPM (Figure 2C); therefore, the results obtained with this membrane composition were close to the ones obtained with 100% silicone (Figure 2A, Table 1).

The further addition of IPM (30% (Figure 2D, Table 1) and 50% (Figure 2E) into silicone) increased both permeability range and permeability values of compounds having poor permeation through human skin, while no significant changes were observed for other compounds. The increased affinity toward IPM of solutes characterized by low permeation through silicone might explain the bigger permeation values obtained with silicone−IPM mixtures for these compounds. Addition of 50% of IPM into silicone decreased the overall permeability range of all tested compounds, and therefore, smaller discrimination according to the compounds' log $K_p$ values was observed. Indeed, the plot of log $P_e$ versus log $K_p$ with this membrane composition is close to the one obtained with 100% of IPM (Figure 2B, Table 1).

Finally, the membrane composition containing 30% IPM into silicone showed the best results in terms of both permeability coefficients discrimination toward human skin permeability and correlation between these two parameters. As a consequence, this membrane composition was chosen as a new artificial membrane for the fast prediction of passive human skin permeability.

To better evaluate the potential of this new artificial membrane (defined as PAMPA-skin) to mimic the human skin, an additional set of 11 compounds was tested in permeation studies (Table 2).

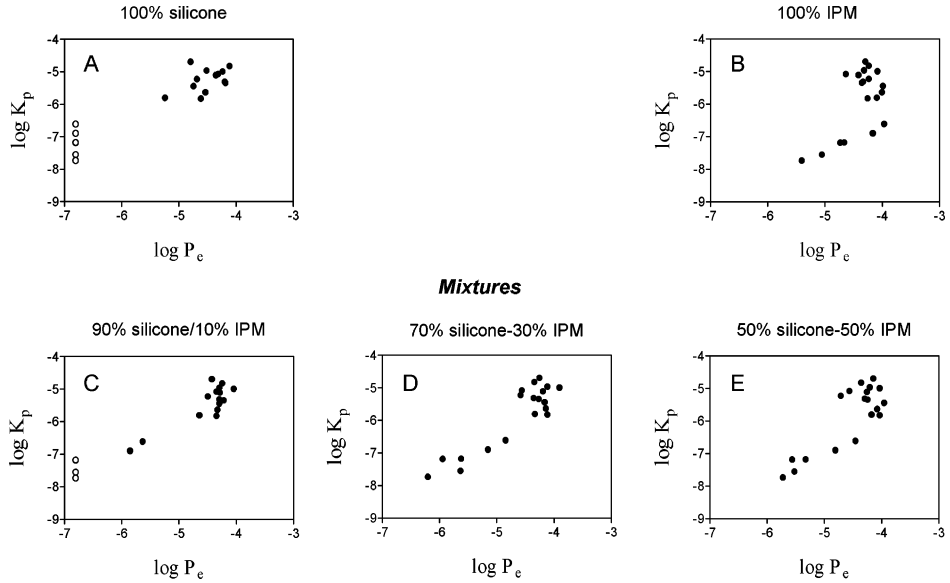


**Figure 2.** Human skin permeability coefficient log $K_p$ vs effective permeability coefficients log $P_e$ determined using different membrane silicone−IPM mixtures (expressed as % v/v): 100% silicone (A); 100% IPM (B); 90% silicone−10% IPM (C); 70% silicone−30% IPM (D); 50% silicone−50% IPM (E). Open circles represent compounds not accurately UV-detectable in the acceptor compartment after the incubation time.

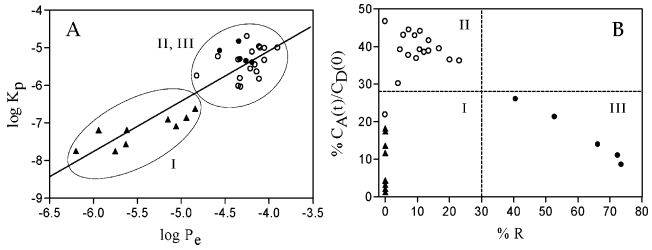


**Figure 3.** (A) Correlation between effective permeability coefficients log $P_e$ determined through 70% silicone−30% IPM membrane and human skin permeability coefficient log $K_p$ for the extended set. The line was obtained by the linear regression equation log $K_p = (1.34 \pm 0.12) \log P_e + (0.28 \pm 0.56)$, where $n = 31$, $r^2 = 0.81$, $s = 0.42$, and $F = 124$. (B) Membrane retention % $R$ and amount of compounds found in the acceptor compartment after 7 h of incubation time (% $C_A(t)/C_D(0)$) using 70% silicone−30% IPM membrane: (○) compounds with log $K_p < -6$ and low or negligible membrane retention; (●) compounds with log $K_p < -6$ and high membrane retention; (▲) compounds with log $K_p \geq -6$.

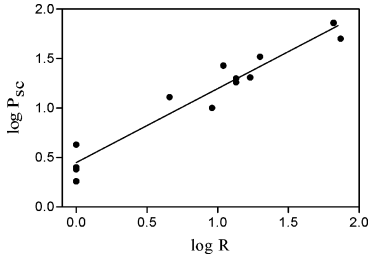


**Figure 4.** Correlation between membrane retention log $R$ and stratum corneum/water partition coefficients log $P_{SC}$. For $R < 1$, i.e., log $R < 0$, log $R = 0$ was attributed. The line was obtained by the linear regression equation log $P_{SC} = (0.75 \pm 0.06) \log R + (0.45 \pm 0.06)$, where $n = 13$, $r^2 = 0.93$, $s = 0.14$, and $F = 159$.

The correlation between log $P_e$ and log $K_p$ obtained with 31 compounds on 70% silicone−30% IPM membrane is shown in Figure 3A. The regression line was obtained using the parameters shown as follows:

$$\log K_p = (1.34 \pm 0.12) \log P_e + (0.28 \pm 0.56) \quad (1)$$

$$n = 31; \quad r^2 = 0.81; \quad s = 0.42; \quad F = 124$$

The statistically reasonable fit ($r^2 = 0.81$) proves that the artificial membrane composition of 70% silicone−30% IPM represents a good model for percutaneous permeation. In addition, the presence of IPM as only an H-bond acceptor group in the artificial membrane is in good agreement with previous results demonstrating that stratum corneum lipids accept H-bonds better than they donate.[44]

**New Information on Skin Permeation Provided by PAMPA-Skin.** A skin reservoir for chemicals has been reported to exist in the stratum corneum for topically applied steroids[45] and for pesticides.[46] When material remaining in the skin is included in the amount of compounds diffused in the receptor compartment, in vitro and in vivo correlations were usually improved.[47] As a consequence, the determination of compound retention in the stratum corneum could be very useful in the development of drugs, in risk assessment studies, and for cosmetics.

In PAMPA it is relatively easy to determine membrane retention, and Figure 3B shows the relationship between membrane retention $R$ and the permeation parameter $C_A(t)/C_D(0)$ for the 31 tested compounds grouped according to their log $K_p$ values. With the new artificial membrane (70% silicone−30% IPM), it is possible to discriminate (1) permeants with lower $K_p$ (log $K_p < -6$) having negligible membrane retention and low permeation ($I$) and (2) compounds with higher $K_p$ (log $K_p \geq -6$) having low or negligible membrane retention and high permeation (**II**) or having high membrane retention and low permeation (**III**). For this group, there is a negative correlation between membrane retention and $C_A(t)/C_D(0)$, meaning that for these compounds the membrane behaves like a trap.

To verify whether the amount of material trapped in the 70% silicone−30% IPM artificial membrane reflects the affinity of compounds toward the stratum corneum barrier, for a limited set of compounds, membrane retention $R$ values were compared to the respective available stratum corneum/water partition coefficient (log $P_{SC}$) values (Table 3, Figure 4). The log $P_{SC}$ physicochemical parameter represents the affinity of compounds for SC relative to the aqueous phase, and this parameter has been used in some dermal absorption models.[48] A positive correlation exists between these two parameters, meaning that

retention on 70% silicone−30% IPM (membrane) is correlated to the compounds' intake in the stratum corneum barrier. These results illustrate that PAMPA-skin is a suitable model for the main barrier properties of the stratum corneum and that a simple determination of compound retention on 70% silicone−30% IPM artificial membrane offers a good estimation of SC/water partition coefficients.

In addition, for tested compounds, octanol/water partition coefficients ($\log P_{oct}$) and alkane/water partition coefficients ($\log P_{alk}$) are worse predictors of stratum corneum partition coefficients ($\log P_{SC}$) ($r^2 = 0.75$ and $r^2 = 0.71$, respectively) than membrane retention on 70% silicone−30% IPM membrane ($\log R$) ($r^2 = 0.93$).

The possibility of discriminating compounds according to their membrane retention and the evidence that the retention in PAMPA-skin reflects the affinity of compounds for stratum corneum provide useful additional information for transdermal drug delivery studies, for the evaluation of the risk associated with dermal contacts with toxic substances, and for cosmetics.

## Conclusion

A good correlation between permeability coefficients through human skin and through the new PAMPA-skin artificial membrane was found. This is the first application of the PAMPA technique for the fast prediction of human skin permeability. Moreover, in addition to $K_p$ predictions, PAMPA-skin provides new insights useful for the assessment of dermal drug delivery, in absorption studies of toxic compounds, and in the cosmetics industry. High permeable compounds through human skin are differentiated by PAMPA-skin into two groups, namely, compounds trapped in the artificial membrane and compounds not retained by the membrane. For a restricted set of compounds, the membrane retention determined in this new in vitro system reflects the affinity of compounds for the stratum corneum. Hence, PAMPA-skin provides a fast estimation of the amount of compounds trapped in the stratum corneum.

## Experimental Section

**Chemicals.** All compounds were purchased from Sigma (division of Fluka Chemie AG, Buchs, Switzerland). DMSO (purity grade, >99.7%) was purchased from Acros Organics (Chemie Brunschwig AG, Basel, Switzerland). Isopropyl myristate (IPM; purity grade. >95%), silicone oil (DC 200), and hexane (purity grade, >99.5%) were purchased from Fluka. The buffers were prepared according to Phoebus software at a fixed ionic strength of 20 mM.

**Permeability Measurements Using the PAMPA Technique.** Permeation experiments were carried out in hydrophobic PVDF 96-well microtiter filter plates (Millipore AG, Volketswil, Switzerland). Each well was coated with 17 $\mu$L of 35% (v/v) liquid membrane dissolved in hexane for 20 min to completely evaporate the solvent. Next, the donor plate was placed on a Teflon acceptor plate (Millipore MSSACCEPTOR) that had been prefilled with 280 $\mu$L of buffer containing 5% DMSO. Then the donor compartments were hydrated with 280 $\mu$L of tested compounds in buffer containing 5% DMSO, and the resulting sandwich was incubated at room temperature under constant shaking (150 rpm). Reference compound concentrations were chosen according to their solubility and UV detection limits (i.e., 100−1875 $\mu$M). Each compound was measured in triplicate or quadruplicate, iso-pH conditions were used (same pH in donor and acceptor compartments), and each compound was measured at a pH value corresponding to an un-ionized fraction ($f_{ui}$) greater than 0.8. The pH of buffer solutions used for tested compounds was defined as follows: pH 2 (NaOH, $H_3PO_4$) for ketoprofen, diclofenac, indomethacin, naproxen, and salicylic acid; pH 3.5 (NaOH, formic acid) for piroxicam; pH 5 (NaOH, AcOH) for phenobarbital and 4-nitrophenol; pH 6 (NaOH, $H_3PO_4$) for phenols and steroids; pH 7 (NaOH, $H_3PO_4$) for isoquinoline, atrazine, 2-nitrophenylenediamine; pH 10 (NaOH, $H_3BO_3$) for nicotine; pH 11.5 (NaOH, $H_3PO_4$) for lidocaine and ephedrine.

After 7 h, the sandwich was disassembled and both donor and acceptor compartments were transferred to a UV quartz plate (Hellma GmbH & Co, Müllheim). UV absorption was measured with a PowerWave (Bio-Tek Instruments, Inc. USA), and the reading was performed at the compounds' $\lambda_{max}$.

To assess membrane stability, electrical resistance measurements were conducted on the filter plate at the end of the incubation time, using an electrometer system especially designed for PAMPA assays (EVOMX and MULTI96, World Precision Instruments, Sarasota FL). Undamaged filters showed electrical resistance values greater than 200 k$\Omega$, and wells with electrical resistance values of ~0.3 k$\Omega$ (indicating the presence of aqueous contacts between the two sides of the filter) were discarded.

**Permeability Measurements Using the Diffusion Cell Method.** Permeability coefficients across silicone membranes were determined using the published method of Geinoz et al.[13] All compounds were purchased from Sigma (division of Fluka Chemie AG, Buchs, Switzerland). The buffers were prepared according to Phoebus software at a fixed ionic strength of 20 mM. Iso-pH conditions were used (same pH in donor and acceptor compartments), and each compound was measured at a pH value corresponding to an un-ionized fraction ($f_{ui}$) greater than 0.8. The pH of buffer solutions used for tested compounds was as follows: pH 2 (NaOH, $H_3PO_4$) for salicylic acid and pH 7 for 2-naphthol and thymol (NaOH, $H_3PO_4$).

**p$K_a$ Measurements.** To ensure a better homogeneity, only p$K_a$ values obtained by the potentiometric method were considered and, for most of the tested compounds, they were taken from the literature. When no data were available, p$K_a$ values were determined by potentiometric titration using a GlpKa apparatus (Sirius Analytical Instruments Ltd., Forest Row, East Sussex, U.K.) equipped with a glass electrode (Sirius) and a temperature probe. All titrations were conducted under nitrogen at 25 ± 0.1 °C[32] and with aqueous solution containing 0.15 M KCl to maintain ionic strength constant.

Because of the relatively low solubility of some compounds in water, the ionization constants of all tested compounds were performed in different methanol−water mixtures (between 25% and 60% w/w in methanol). Then the apparent p$K_a$ values were extrapolated to zero cosolvent by the Yasuda−Shedlovsky procedure.[33] Solutions of compounds (0.5−1 mM) were titrated from pH 2 to pH 12 for basic compounds and ordinary ampholytes and from pH 12 to pH 2 for acidic compounds using HCl (0.5 M) and KOH (0.5 M).

**Permeability Equations.** Equations used to calculate permeability coefficients can be deduced in several ways according to experimental conditions and to the design of the in vitro assay.[34]

When membrane retention cannot be neglected, the effective permeability coefficients $P_e$ (cm/s) was calculated using the following published equation:[34]

$$P_e = -\frac{2.303V_D}{A(t-\tau_{LAG})}\left(\frac{V_A}{V_A+V_D}\right)\log\left[1-\left(\frac{V_A+V_D}{V_D(1-R)}\right)\frac{C_A(t)}{C_D(0)}\right] \quad (2)$$

where $A$ is the filter area (0.3 cm$^2$) multiplied by a nominal porosity of 70% according to the manufacturer, $t$ is the incubation time (s), $\tau_{LAG}$ is the steady-state time (s), i.e., the time needed for the permeant's concentration gradient to become stabilized, $V_A$ and $V_D$ are respectively the volumes in the acceptor and the donor wells (0.28 cm$^3$), $C_A(t)$ is the concentration of the compound (mol cm-$^3$) in the acceptor well at time $t$, and $C_D(0)$ is the concentration of the compound (mol cm$^{-3}$) in the donor well at time 0. $R$ is the retention factor defined as the mole fraction that is lost in the membrane and in the microplates (i.e., filters and plate materials):

$$R = 1 - \frac{C_D(t)}{C_D(0)} - \frac{V_A}{V_D}\frac{C_A(t)}{C_D(0)} \quad (3)$$

where $C_A(t)/C_D(0)$ represents the amount of compound that reached

the acceptor compartment after incubation time $t$ (for $V_A = V_D$). Steady-state times ($\tau_{LAG}$) to saturate the membranes in PAMPA are short relative to the total permeation time (~20 min with unstirred plates),[35] and for this reason they were considered negligible in this study.

As a first approximation, permeation was attributed to the neutral species alone, particularly when the fraction un-ionized is not too low (i.e., $f_{ui} > 0.1$). Consequently, the effective permeability coefficients $P_e$ were divided by the un-ionized fraction ($f_{ui}$).[36] For compounds with a single acidic or basic functional group, this parameter is correlated with the dissociation constant (p$K_a$) and the pH of the two compartments is given by

$$f_{ui} = \frac{1}{1 + 10^g} \tag{4}$$

where $g = (\text{pH} - \text{p}K_a)$ for acids and $g = (\text{p}K_a - \text{pH})$ for bases.

**Adsorption on PVDF Filters and Plates Materials.** Membrane retention has been defined as the amount of compounds that is not found in the acceptor or in the donor compartments after the incubation time.[37] Hence, retention factor $R$ includes artificial membrane retention and, if present, adsorption on plate material and on PVDF filters. To verify the presence of adsorption on the system used, a PAMPA assay was done without coating the PVDF filters with artificial membrane. Results showed that adsorption on plate material and on PVDF filters was negligible for tested compounds except for five lipophilic compounds that presented about 10% of adsorption (data not shown). Additional tests on PVDF filters without plate material demonstrated that these compounds were adsorbed on these filters. However, on PVDF coated filters, the surface of PVDF accessible to the compounds is much lower than with PVDF uncoated filters and, accordingly, the overall adsorption in the presence of artificial membrane must be lower than adsorption observed without membrane for tested compounds. For this reason, in this study no corrections for nonspecific adsorption were applied.

**Skin Absorption Database.** Permeability coefficients data ($K_p$, cm/s) used in this study were measured in vitro through human skin from aqueous vehicles, and they refer to permeation of neutral species. When compounds were partially ionized at experimental pH, permeability coefficients were divided by the un-ionized fraction present in solution. Most of the permeability coefficients were collected from a fully validated database.[36] Note that when more than one value was available for a compound in this database, values that also appeared in the Flynn database were chosen. For steroids, the most recent data from Johnson et al.[38] and Mitragori et al.,[39] which have been shown to be preferable to the older values,[40] were used. For caffeine and lidocaine, data were also taken from Mitragori et al.[39] and Johnson et al.,[41] respectively.

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**Supporting Information Available:** Membrane retention ($R$) and $C_A(t)/C_D(0)$ values obtained through 100% silicone membrane and 100% IPM membrane for the first 19 tested compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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